

WHAT IS CLAIMED IS:

1. A retroviral vector for carrying a target gene specific insert into a cell in order to modify the expression of a target gene, comprising:

5 (a) a promoter;
(b) a polylinker region;
(c) a target gene specific insert comprising double stranded RNA, wherein said double
stranded RNA comprises a sense portion that is complementary to a portion of the antisense strand of
the target gene, and an antisense portion that is complementary to the sense portion, so that the sense
10 portion and antisense portion anneal, and the double stranded RNA folds back upon itself.

2. The retroviral vector of Claim 1, wherein the promoter is selected from the group consisting of

(a) a U6 promoter sequence of:

ttcccatgattcctcatattgcatacagatacaggcttagagagataattagaattaatttgactgtaaacacaaagatattgtacaaaatacgtg
acgttagaaagtaataattcttggtagttgcagttttaaaattatgttttaaaatggactatcatatgcttaccgttaacttgcatttcatttgcc
tttatatatcttgtgaaaggacgaaacacccg (SEQ ID NO:7); and

(b) an H1 promoter (SEQ ID NO:14).

3. The retroviral vector of Claim 1, wherein the polylinker region comprises a nucleotide sequence selected from the group consisting of:

- (a) aattc gactggcacagcctccagg ttcaagaga cctggaggctgtgccagtc tttt ggaa a (SEQ ID NO:1)
- (b) aattc gctggactccttgcatg ttcaagaga catgcaaaggagtcccagc tttt ggaa a (SEQ ID NO:2);
- (c) gatcc gactggcacagcctccagg ttcaagaga cctggaggctgtgccagtc tttt ggaa a (SEQ ID NO:3);
- (d) gatcc gctggactccttgcatg ttcaagaga catgcaaaggagtcccagc tttt ggaa a (SEQ ID NO:4)
- (e) aattc gactccagtggtaatctac ttcaagaga gtagattaccactggagtc tttt ggaa a (SEQ ID NO:5); and
- (f) gatcc gactccagtggtaatctac ttcaagaga gtagattaccactggagtc tttt ggaa a (SEQ ID NO:6).

4. The retroviral vector of Claim 1, wherein the sense and antisense regions of the target gene specific insert each comprise a length of 19-30 nucleotides.

5. The retroviral vector of Claim 4, wherein the sense and antisense regions of the target gene specific insert each comprise a length of 19-25 nucleotides.

30 6. The retroviral vector of Claim 5, wherein the sense and antisense regions of the target gene specific insert each comprise a length of 19-23 nucleotides.

7. The retroviral vector of Claim 1, wherein the target gene is an oncogene.

8. The retroviral vector of Claim 1, wherein the retroviral vector is a Murine Stem Cell Virus (MSCV).

35 9. The retroviral vector of Claim 1, wherein the retroviral vector is a modified Lentivirus
in which:

- (a) the endogenous CMV promoter of the Lentivirus has been removed; and
- (b) a REV element that binds to a REV response element (RRE) is inserted.

10. A cell infected with the retroviral vector of Claim 1, wherein said cell has said target gene in its genome.

5 11. A modified Lentivirus vector for carrying double stranded RNA into a cell in order to modify the expression of a target gene, wherein:

(a) the endogenous CMV promoter of the Lentivirus has been removed, said modified

Lentivirus vector comprising:

(i) a REV element that binds to a REV response element (RRE) is inserted;

10 (ii) a U6 promoter sequence of

ttcccatgattcctcatattgcatacatacataaggctgttagagagataattttactgtaaacacaaagatattgtacaaaatacgtg
acgttagaaagaataattctggtagttgcagttttaaaattatgtttttaaaatggactatcatatgcttaccgtaacttggaaagtatttcgatttgc
tttatatatcttggaaaggacaaacacccg (SEQ ID NO:7); and

(iii) a polylinker region;

15 wherein said double stranded RNA comprises a sense portion that is complementary a portion of the antisense strand of the target gene, and an antisense portion that is complementary to the sense portion so that the sense portion and antisense portion anneal, and the double stranded RNA folds back upon itself.

20 12. The modified Lentivirus vector of Claim 11, wherein said polylinker region is selected from the group consisting of:

(a) aattc gactggcacagcctccagg ttcaagaga cctggaggctgtgccagtc tttt ggaa a (SEQ ID NO:1)

(b) aattc gctgggactccttgcatg ttcaagaga catgcaaaggagtcccagc tttt ggaa a (SEQ ID NO:2);

(c) gatcc gactggcacagcctccagg ttcaagaga cctggaggctgtgccagtc tttt ggaa a (SEQ ID NO:3);

(d) gatcc gctgggactccttgcatg ttcaagaga catgcaaaggagtcccagc tttt ggaa a (SEQ ID NO:4)

25 (e) aattc gactccagtggtaatctac ttcaagaga gtagattaccactggagtc tttt ggaa a (SEQ ID NO:5);

and

(f) gatcc gactccagtggtaatctac ttcaagaga gtagattaccactggagtc tttt ggaa a (SEQ ID NO:6).

13. The modified Lentivirus vector of Claim 12, further comprising a reporter gene.

14. The modified Lentivirus vector of Claim 13, wherein said reporter gene is selected 30 from the group consisting of Blasti and hrGFP.

15. The modified Lentivirus vector of Claim 14, selected from the group consisting of:

(a) pLenti-U6-Blasti, which comprises the nucleotide sequence of SEQ ID NO:8; and

(b) pLenti-U6-hrGFP, which comprises the nucleotide sequence of SEQ ID NO:9.

16. A Murine Stem Cell Virus (MSCV) vector for carrying double stranded RNA into a 35 cell in order to modify the expression of a target gene, comprising:

- (a) a promoter; and
- (b) a polylinker region,

wherein said double stranded RNA comprises a sense portion that is complementary a portion of the antisense strand of the target gene, and an antisense portion that is complementary to the sense portion so that the sense portion and antisense portion anneal, and the double stranded RNA folds back upon itself.

17. The MCSV vector of claim 26, wherein said promoter is the U6 promoter sequence of:

10 ttcccatgattcctcatattgcatacgcataaggctgttagagagataattagaattaattgtactgtaaacacaaagatattgtacaaaatacgtg
acgttagaaagtaataattcttggtagttgcagttttaaaattatgtttaaaatggactatcatatgcttaccgtacttgcatttcgattcttgcc
tttatatatcttggaaaggacgaaacacccg (SEQ ID NO:7).

18. The MCSV vector of Claim 17, wherein said polylinker region is selected from the group consisting of :

15 (a) aattc gactggcacagcctccagg ttcaagaga cctggaggctgtgccagtc tttt ggaa a (SEQ ID NO:1)
(b) aattc gctggactcccttgcatg ttcaagaga catcaaaggagtcggcagc tttt ggaa a (SEQ ID NO:2);
(c) gatcc gactggcacagcctccagg ttcaagaga cctggaggctgtgccagtc tttt ggaa a (SEQ ID NO:3);
(d) gatcc gctggactcccttgcatg ttcaagaga catcaaaggagtcggcagc tttt ggaa a (SEQ ID NO:4)
(e) aattc gactccagtggtaatctac ttcaagaga gtagattaccactggagtc tttt ggaa a (SEQ ID NO:5); and
(f) gatcc gactccagtggtaatctac ttcaagaga gtagattaccactggagtc tttt ggaa a (SEQ ID NO:6).

19. The MSCV vector of Claim 18, further comprising a reporter gene.

20. The MSCV vector of Claim 19, wherein said reporter gene is selected from the group consisting of Hygro, Puro, and hrGFP.

21. The MSCV vector of Claim 20, wherein said MSCV vector is selected from the group consisting of:

25 (a) MSCV-U6-Hygro, which comprises the nucleotide sequence of SEQ ID NO:10;
(b) MSCV-U6-Puro, which comprises the nucleotide sequence of SEQ ID NO:11; and
(c) MSCV-U6-hrGFP, which comprises the nucleotide sequence of SEQ ID NO:12.

22. A cell infected with the MSCV vector of Claim 16.